

We claim:

1. A test plate for fluorescence imaging systems comprising:
  - a) a surface comprising at least a first chemical group for binding;
  - b) fluorescent microbeads, wherein the fluorescent microbeads comprise at  
5 least a second chemical group that is bound to the at least first chemical group on the surface; and
  - c) a polymeric layer in which the fluorescent microbeads are embedded.
2. The test plate of Claim 1, wherein the polymeric layer comprises a polymer selected from the group consisting of polyurethane, polyacrylate, polysilicones,  
10 polyglycols, and polyvinyl alcohol.
3. The test plate of Claim 1, wherein the at least first chemical group and the at least second chemical group are covalently coupled.
4. The test plate of Claim 1 wherein the at least first chemical group comprises a reactive amine group.
- 15 5. The test plate of Claim 4 wherein the at least first chemical group comprising a reactive amine group is selected from the group consisting of collagen I, bovine serum albumin, fibronectin, laminin, fragments thereof, and organosilanes.
6. The test plate of Claim 4 wherein the at least second chemical group comprises a chemical group selected from the group consisting of carboxylate groups, amide groups,  
20 or sulfhydryl groups.
7. The test plate of Claim 4 wherein the at least second chemical group comprises a carboxylate group.
8. The test plate of Claim 1, wherein the fluorescent microbeads further comprise at least a third chemical group.
- 25 9. The test plate of claim 1 wherein the polymeric layer is doped with a fluorophore that is optically distinguishable from the fluorescent microbead.
10. The test plate of claim 1 wherein the fluorescent microbeads comprise fluorescent microbeads of different sizes.
11. The test plate of claim 1 wherein the fluorescent microbeads comprise fluorescent  
30 microbeads of uniform size.

12. The test plate of claim 1 wherein the fluorescent microbeads in total comprise two or more fluorophores, and where the two or more fluorophores are optically distinguishable.

13. The test plate of claim 1 wherein the polymer layer comprises a series of polymer islands.

14. The test plate of Claim 1 wherein the surface comprises wells and wherein the fluorescent microbeads are located within the wells.

15 The test plate of Claim 14 wherein the fluorescent microbeads are monodispersed in the well.

16 A method of making a fluorescence imaging system test plate comprising:

a) providing a surface comprising at least a first chemical group;

b) providing fluorescent microbeads, wherein the fluorescent microbeads comprise at least a second chemical group that is capable of binding to the first chemical group;

c) contacting the surface with the fluorescent microbeads under conditions to permit binding of the at least first chemical group and the at least second chemical group; and

d) adding a polymeric layer to the surface, wherein the polymeric layer is selected from the group consisting of polyurethane, polyacrylate, polysilicones, polyglycols, and polyvinyl alcohol, wherein the fluorescent microbeads are embedded in the polymeric layer.

17. The method of Claim 16 wherein the method further comprises drying the test plate prior to the addition of the polymeric layer.

18. The method of Claim 16, wherein the at least first chemical group and the at least second chemical group are capable of covalently coupling each other.

19. The method of claim 16 wherein the polymeric layer comprises a polymer selected from the group consisting of polyurethane, polyacrylate, polysilicones, polyglycols, and polyvinyl alcohol.

20. The method of Claim 16 wherein the method of contacting is selected from the group consisting of transferring the fluorescent microbeads to the surface and allowing

the microbeads to settle to the surface by gravity, and transferring the fluorescent microbeads to the surface and centrifuging the test plates.

21 The method of Claim 16 wherein the surface comprises wells, and wherein the fluorescent microbeads are contacted with the wells of the surface.

5 22. A method for testing a fluorescence imaging system comprising:

a) providing the test plate of claim 1 with fluorescent microbeads fixed on a surface of the test plate;

b) acquiring fluorescent images from the fluorescent microbeads;

c) making a measurement of a property of the fluorescent images selected

10 from the group consisting of: intensity, area, density, and distribution;

d) comparing the values of the measured property to a known value; and

e) adjusting a parameter of the fluorescence imaging system as necessary based on the values of the measured property.